Iraqi Propolis effective in avoiding deviations of gut rat's homeostasis exposed to AFB-1

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Abstract

Background: Consumption of food contaminated with Aflatoxin-B1(AF-B1), caused deleterious effects on different body's systems ,specially GIT in direct and indirect manners. To investigate the protective role of propolis against AF-B1 effects on rats gut, 24 male rats divided into equal 4 groups: group (C)was control, second group (AF) received Aflatoxin-B1 (0.025mg/kg), third group(P) received Propolis in dose (50 mg /kg BW) and the fourth group(AFP) received Aflatoxin-B1 (0.025 mg/ 1kg BW) + Propolis (50 mg / 1kg BW).after 60 days of experiment , intestinal D-zylose adsorption and histomorphological changes were measured

Results: Results revealed that intestinal absorption function of D-Xylose sugar was reduced in AFB-1 exposed rats , meanwhile Propolis improve D-xylose absorption in rats exposed to the AFB-1. Histomorphological measurements include mucosa thickness, Villus high, Crypts depth, Villus/crypt ratio, and goblet cells density in the three portions of small intestine decreased significantly in AF group and increased significantly in P group and kept semi-normal in AFP group in comparison with the control group. Analysis of light microscopic photograph revealed that administration of aflatoxin-B1 cause deleterious changes in stomach, and intestinal tissues, while Propolis was efficient in improving these changes to normal condition.

Conclusions: Intestinal absorptive function and histomorphological measurements were altered by Aflatoxin-B1, and the duodenum was the most affected part from the intestine. Results of propolis showed a high activity to improve all the above measurements and counter the deleterious effect of Aflatoxin-B1 to normal situation.

Keywords: propolis; Aflatoxin-B1; D-xyloze test; gut homeostasis

فعالية البروبولس العراقي في تجنب انحرافات بيئة امعاء الجرذان المعرضة للافلاتوكسين

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المستخلص

تسبب تناول الغذاء الملوث بافلاتوكسين بتأثيارت ضارة على اجهزة الجسم وبالخصوص القناة الهضمية بشكل مباشر وغير مباشر . ولغرض تحقق من دور البروبولس ضد تأثيرات الافلاتوكسين في معوي الجرذان . تم توزيع 24 جرذ ذكر الى اربع مجاميع متساوية حيث مجموعة سيطرة ومجموع ثانية اعطيت افلاتوكسين

(0,025 ملغم / كغم وزن جسم ومجموعة ثالثة اعطيت بروبولس 50 ملغم / كغم ومجموعة رابعة اعطيت 0,025 ملغم الفلاتوكسين + 50 ملغم بروبولس/ كغم . بعد 60 يوما تم اجراء فحص الدي زايلوز وقياس التشكيلات النسيجية للامعاء

بنت النتائج ان وظيفة ألأمعاء الامتصاصية انخفضت في جرذان مجموعة الافلاتوكسين بينما اثبت البروبولس امتصاص الزايلوز في الجرذان المتناولة للافلاتوكسيننوتشمل القياسات الهيستومورفولوجية سماكة الغشاء المخاطي، وقلوب القاع، وعمق الخبايا، ونسبة الفيلوس / الكريبت، وكثافة الخلايا الكأسية في الأجزاء الثلاثة من الأمعاء الدقيقة انخفضت معنويا في المجموعة أف وزادت بشكل معنوي في المجموعة P وأبقت شبه طبيعية في مجموعة أف مقارنة مع المجموعة الضابطة. كشف تحليل الصورة المجهرية الخفيفة أن إدارة الأفلاتوكسين−B1 تسبب تغيرات ضارة في المعدة، والأنسجة المعوية، في حين أن دنج كان فعالا في تحسين هذه التغيرات في حالة طبيعية.

الاستنتاجات: تم تغيير وظيفة الامتصاص المعوية والقياسات الهيستومورفولوجية من قبل أفلاتوكسين−B1 ، وكان الاثنى عشر الجزء الأكثر تضررا من الأمعاء. وأظهرت نتائج الدنج نشاطا كبيرا لتحسين جميع القياسات المذكورة أعلاه ومواجهة تأثير ضار أفلاتوكسين−B1 إلى الوضع الطبيعي .

Introduction

Propolis , is a resinous wax-produced by the honey bees from plant exudates. Propolis showed an effective roles against many pathogenic microorganisms and counteract the effects of toxic material [2] and has powerful antioxidant compound, containing high quantities and different types of flavonoids, terpens and phenolic groups[9,13]. Propolis as a crude or it's extracted active compounds had been investigated for improvement of intestinal functional and structural characteristics. Propolis strength the barrier of colon epithelia of rats suggesting an activating specific pathways for synthesis and secretion of mucus and renewal of intestinal mucosa [21].

Aflatoxins are by-products of a soil-borne fungus which called *Aspergillus*, these toxins contaminate different food stuffs of human and animals. Consumption of food contaminated with Aflatoxin-B1, caused deleterious effects on different body's systems, specially gastrointestinal tract (GIT) in direct and indirect manners[39]. Intestinal mucosa is a target tissue for mycotoxin effects , since it is the site were these toxins are primarily absorbed so excrete deleterious effects on epithelial cells. For the importance of intestinal mucosa integrity and functionally, recent research had focused on studying the intestinal histomorphological changes in AF-B1, [22; 24]. AF-B1 exposing caused harmful effects on hematopoietic system result in anemia [8] . Furthermore AF-b1deleterious effects induced the generation of intracellular reactive oxygen species (ROS), leading to oxidative stress which can cause cellular damage [11]. These deleterious effects caused by intestinal contaminated food by AF-B1 can be countered by natural materials, obtained from plants Curcumin [10] or lemon grass oils [36]. The survey of previous literature related to this study was observed lake of recourses which interested in studding of effectiveness of Propolis, as a natural prod-

uct against AF-B1 effects, based on the forgoing it designed the current study, to investigate the Propolis activity in ameliorating the deleterious physiological and structural aspects of intestine in rats caused by AF-B1.

Material and Methods

Experimental animals and design:

The present study was conducted in the department of physiology and pharmacology in college of veterinary medicine /Baghdad university, and designed according to the Research Ethic standards. Twenty four adult male rats were divided into 4 equal groups:1st (C) control group; 2nd (P) were given daily by oral gavage (50 mg /kg body weight) of propolis ;3rd (Af) this group were given Aflatoxin (0.025mg/kg body weight); 4th (AFP) this group will be given by oral gavage (50 mg /kg body weight) of propolis + Aflatoxin (0.025mg/kg body weight). They all were kept under suitable condition of (21-25 C) in an air-condition room and fed freely with standard pellet diet. All applications were administered daily for 60 consecutive days. At The end of the experiment D-xylose absorbtion test was performed to all animals.

Dosage of Propolis and Aflatoxin-B1

Crude propolis obtained from local markets as Iraqi propolis and the soaking method (maceration) was used to prepare propolis extract ,the dosage was designed according to previous studies by [32] The dosage of aflatoxin was chosen according to the effective dosages stated by previous studies [37; 34] .One milligram of AF-B1 was dissolved in 40ml of distilled water. The solution was used for administration of AF-B1 through an oral gavage in a dose 25µm/kg body weight.

Intestinal D-zylose absorbtion:

After 60 days of experiment, all experimental animals were food and water deprived over night. Orally D-Xylose solution((100mg/ 1m / 100g B.W.),)was gavaged in a dose of 100mg/ 1m / 100g B.W . Each animal was hold in a metabolic cage for 5 hours for urine collection. Blood and urine D-xylose concentration then measured by using of the standard colorimetric method [20] , which depends on measurement of density of the brown color produced from the reaction of D-xylose with O- toluidine in presence of acetic acid and heat.

$$\text{D-xylose concentration (g\l)} = \frac{\text{Mean absorbance of test}}{\text{Mean absorbance of Standard}} \text{ X Concentration of Standard}$$

Intestinal absorption of D-xylose % was calculated according to [23] .

$$\text{Absorbed D-xylose \%.} = \frac{\text{Amount of D-xylose in (g) excreted in urine}}{\text{Amount of ingested D-xylose in (g)}} \text{X100}$$

Amount of D-xylose excreted in urine (g) = (Concentration of D-xylose in urine(g/l)×urine volume (ml))/1000.

Intestinal Histomorphological measurements

Immediately after animal sacrifice specimens were collected from colonic and jejunum and were fixed in 10% formalin in phosphate buffered saline, embedded in paraffin and cut into 4 μm sections. In hematoxilin/eoisin staining of series sections of different parts of intestinal wall, the mucosal thickness, villus high, crypt depth and villus high / crypt depth ratio were measured at low power (10 and 250X) using a Winjoe ocular micrometer . These variables were measured in 30 wells of different sections for each animal.

Density of Goblet Cell:

Goblet cells were identified as acidic mucin secreting cells and were assessed by staining with Periodic acid-Schiff (PAS), according to [14]. Determination of the number of goblet cells limited by cells with PAS positive stained along the villi by light microscope, as cell per 100 epithelia cells. Goblet cells were measured in twenty field for each intestinal sample/animal. Only the goblet cells found on the edge of the villas were counted for the density determination

Statistical Analysis. Complicated randomized design was applied (CRD) to study the effect of the transactions studied in different qualities and compared the moral differences between the averages by Duncan polynomial test, and used SAS(2010) program in the statistical analysis .

Results and discussion

Stomach and intestinal weight / Body weight ratio:

The results obtained from the experiment in table-1 referred to the organs weight/ body weight. There was a tendency to increase intestine, stomach and liver weights/ body weight ratio in AF-B1 group (5.75±0.12, 0.733±0.04 and 0.03147±0.003) as compared with other groups. The increase of intestine/B.W. and stomach/B.W. ratio denoted in the present study may be explained by the histopathological changes occurred in tissues of these organs. Increase in mass of organs under AF-B1 orally exposure attributable to the hasty loading of polymorphoneuclear cells infiltration in addition to the sub mucosal thickness. These changes were less in groups which received Propolis and had a semi normal organ mass.

Table-1 : Protective Effect of Propolis on intestine weight/ body weight, stomach weight/ body weight in male rats with Aflatoxin induced anemia for 60 days (Mean ± SE. No=6.)

Treatments	Intestine weight/ B.W.	Stomach weight/B.W.
C	4.43 ± 0.18^{b}	0.616 ± 0.03^{b}
AF	5.75 ± 0.12^{a}	0.733 ± 0.04^{ab}
P	4.25 ± 0.19^{b}	0.683 ± 0.04^{ab}
AFP	4.83 ± 0.30^{b}	0.650 ± 0.02^{a}
LSD	0.62	0.102

C = received distilled water AF= received Aflatoxin-B1 (25µm//kg) P= received Propolis in dose (50 mg /kg BW) AFP= Received Aflatoxin-B1 (0.025 mg/ 1kg BW) + Propolis (50 mg / 1kg BW) B.W.= body weight . Small letter = indicate significant ($P<0.05$) differences between groups (columns).

Intestinal absorption of D-xylose:

D-xylose intestinal absorption as shown in table-2 the amount of excreted D-xylose and the calculated intestinal absorption(%) decreased in AF and AFP groups(0.012±0.001, 0.020±0.0007) and increased ($P<0.05$)in propolis group (0.037±0.002) in comparison with control (0.029±0.001). D- xylose is a pentose sugar , it doesn't found in significant amounts in blood. When it was given orally, approximately 60% is passively absorbed in the proximal small intestine, and most of it is subsequently excreted by the kidneys within limited time [35] . For many decades D-xylose absorption is considered as an investigative test study for small intestinal absorptive function [19] .

Certainly, the decreased percentage of D-xylose absorption in AF group, a foregone conclusion for the deleterious effects of Aflatoxin-B1 on intestinal mucosa integrity and bioavailability mentioned above. The increase in D-xylose absorption denoted in rats received propolis, reflected the positive activity of propolis on the absorptive function of intestinal mucosa . This could be explained by the increased absorptive surface length caused by an increase in villus height scored in the present study, since absorption of D-xylose depends on the length of the absorptive surface determined by villus length in which nutrient absorption occurs. On the other hand, studies have shown that propolis increase in mononononucleated cells with moderate goblet cells hyperplasia of lymphoid tissue and eosinophilia reflect the protective role of propolis against toxic materials effects on gut mucosa like Alcl3 [6] , these benifiet effects could be attributed to the highly antioxidant, antimicrobial, antifungal activities of propoli[7; 8; 9; 17] . The amount of D-xylose absorbed depends primarily on the integrity of the duodenum and jejunum. However, diseases which reduce the absorptive intestinal surface such as inflammatory are associated with abnormally low D-xylose excretion in the urine as well as with decreased serum D-xylose concentration[23; 5].

Table-2 : Protective Role of Propolis on given (gm), D- xylose excreted (gm) and intestinal absorption(%) in male rats with Aflatoxin induced anemia for 60 days (Mean ± SE. No=6.)

Treatments	Given (gm)	Excreted (gm)	Intestinal absorption(%)
C	0.421±0.0209[a]	0.0695±0.0007[b]	0.029±0.001[b]
AF	0.391±0.0343[a]	0.0323±0.001[d]	0.012±0.001[d]
P	0.413±0.0289[a]	0.0908±0.0007[a]	0.037±0.002[a]
AFP	0.401±0.0127[a]	0.0511±0.001[c]	0.020±0.0007[c]
LSD	0.1271	0.0026	0.005

C = received distilled water AF= received Aflatoxin-B1 (25µm//kg) P= received Propolis in dose (50 mg /kg BW) AFP= Received Aflatoxin-B1 (0.025 mg/ 1kg BW) + Propolis (50 mg / 1kg BW). Small letter = indicate significant (P<0.05) differences between groups (columns).

Intestinal histomorphological changes

significantly in P group (1200± 209.16) with semi normal values in AFP and C (770± 50.86, 925± 63.73) respectively. In jejunum and ileum the highest villus was recorded in P group (1075± 111.80). Crypt depth (µg) in duodenum(106± 4.00) in AF group decreased more than the other parts jejunum (260± 18.70) and ileum (164± 15.68). Beneficial effects of Propolis against AF-B1 on crypt depth resembled by increasing in layers of crypts (Figures 1&2) with normal single crypt depth. The ratio of villus/ crypt depth increased significantly in duodenum of P & AFP groups (7.12±1.53, 7.00±0.33) at the same time it decreased in AF group (4.10± 0.36) in comparison with C group (4.98± 0.40). In jejunum and ileum, this ratio showed parallel manner with constant results according to results of villus high and crypt depth as shown in figures-1and 2 Light microscopic evaluation of intestinal mucosa revealed that the duodenum was among the most affected parts of

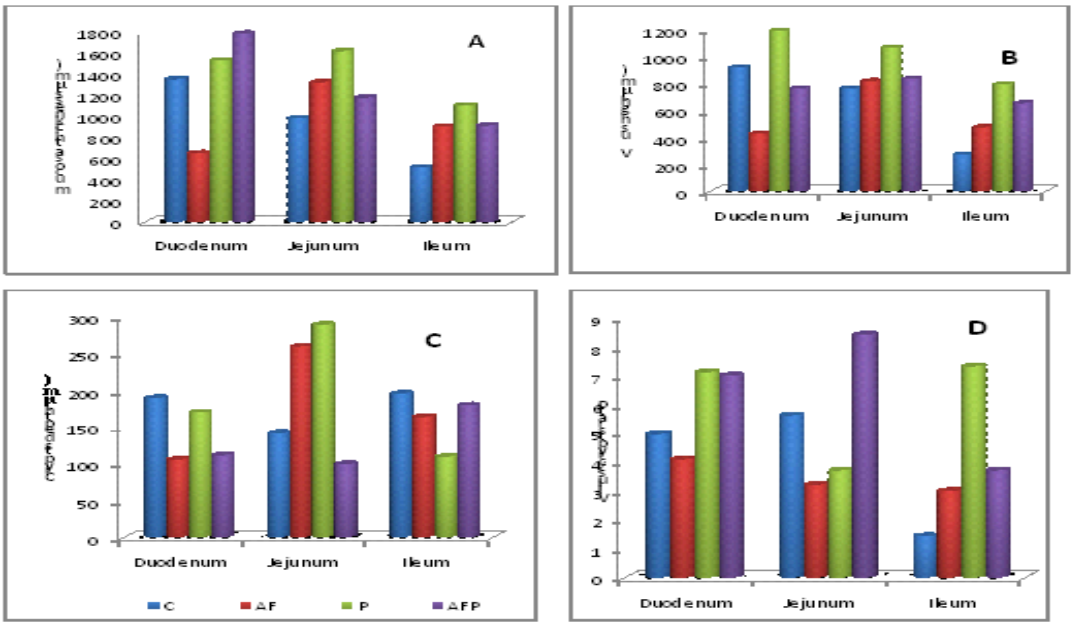


Figure -1: Protective Role of Propolis on: A: mucosa thickness(µm),B: Villus height (µm), C:Crypt depth(µm), and D: villus /crypt ratio in different parts of the small intestine (duodenum, jejunum, ileum) in male rats with Aflatoxin-B1 induced anemia for 60 days C = received distilled water AF= received Aflatoxin-B1 (25µm//kg) P= received Propolis in dose (50 mg /kg BW) AFP= Received Aflatoxin-B1 (0.025 mg/ 1kg BW) + Propolis (50 mg / 1kg BW).. (n=120).

the intestine with aflatoxin , there was reduction in all intestinal histomorphological measurements. The duodenal damaged could be explained by many causes, first of them is that Aflatoxin-B1 is absorbed efficiently from the duodenum [25]. Second, duodenum has the suitable contents for activating the Aflatoxin-B1 mutagenicity due to its neutralized PH and pancreatic fluid [26]. On the contrary to the changes of duodenum ,the jejunum and ileum histomorphological measurements increased in all experimental groups , reflecting the non mutagenic effects of Aflatoxin-B1 on these portions. This observation had been noticed by other researchers [28]. The different histomorphological responses to Aflatoxin-B1 differs with nature and chemical composition of the intestinal content,feeding rats with , propolis showed an ameliorative effects of these histomorphological measurements as a result to activity of its bioactive components such as caffeic acid, flavonoids and

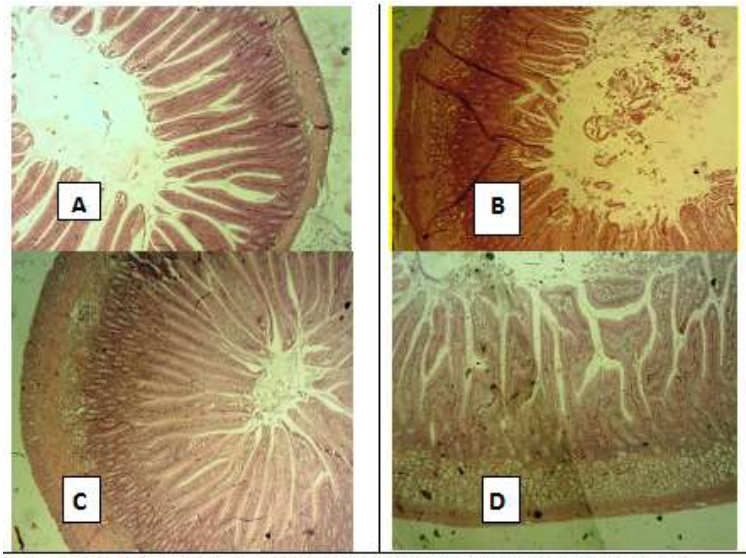
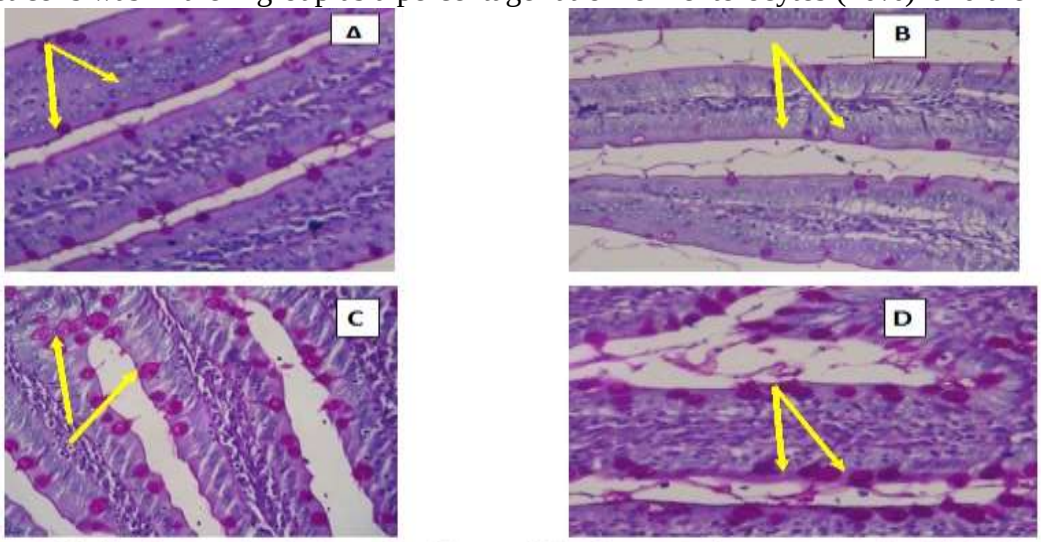


Figure -2 : Light microscopic photograph of duodenum intestinal cross section in A: control showing normal long villi. B : AF group, showing short, necrotic, lacerated villi. C: P group, long, twisted, crowded villi . D: AFP group showing long, branched, semi normal villi. H&E stain,(100X)

the polyphenolic compounds which are very active antioxidants which acts as anti-inflammatory and decreased the mucosal damage and avoid subtotal atrophic changes of intestinal villi [15,18].
The results obtained in this experiment shown in Figures-3and 4 revealed that the higher density of goblet cells was in the P group as a percentage ratio from enterocytes (20%) and the lesser one was



re-3: Light microscopic photograph of ileum vili in: A: control group, normal goblet cells ribution. B: AF group, few and small goblet cells. C: in P group, large and abundant goblet distribution. D: AFP group. goblet cells increased in size and number. Pass stain,(400X)

in AF group (12%). Meanwhile, the denisity of goblet cells in AFP ; 13.5% as compared to the control group(15.5%) .The number of periodic acid- Schiff positive cells were obvious clearly in P and AFP groups , and as much in AF and control groups. Goblet cells are produced in the crypts of the intestinal tract and over a period of approximately three days goblet cells migrate up along the sides of the villi, towards the villi tip where they will eventually be sloughed and released into the intestinal lumen , these goblet cells are replaced in a continuous manner[42]. Aflatoxin-B1 caused oxidative stress to intestinal tissue cells ,disruption of these cells and loss of the proper integrity in addition to other effects like inflammatory and mutagenic alterations which dependently leads to sloughing of mucosal layer cells including the goblet cells. Furthermore it may cause depression in the production of cytokines which regulates the differentiation of enterocytes into goblet cells. One or all of the above mechanisms involved in reducing goblet cells in Aflatoxin-B1 exposed rats. Certainly in our experiment, there was a tendency to an restored goblet cells to normal levels and size when propolis was administered to Aflatoxin-B1 exposed rats. It had been found that intestinal crypt depth (but not villus length) increased linearly with increasing Aflatoxin-B1 concentration [24].

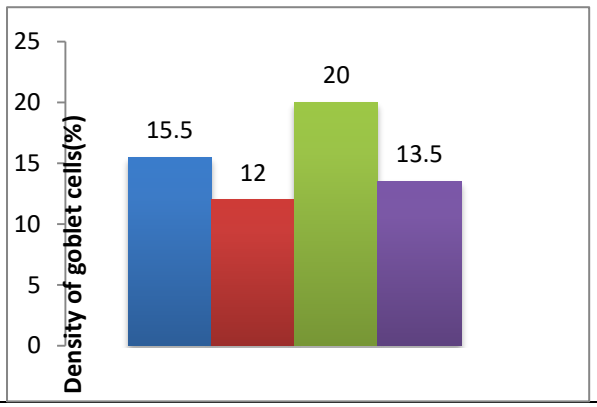


Figure-4 : Protective Role of Propolis on goblet cells density(%) in ileum of male rats with Aflatoxin induced anemia for 60 days.C = received distilled water AF= received Aflatoxin-B1 (25µm//kg) P= received Propolis in dose (50 mg /kg BW) AFP= Received Aflatoxin-B1 (0.025 mg/ 1kg BW) + Propolis (50 mg / 1kg BW) B.W.= body weight. (no=80).

Histopathological changes of stomach and intestine

The light microscopic images of ileum sections (Figure-5) revealed that AF-B1 orally administered rats were suffering from severe irritant lesions leads to superficial sloughing of intestinal villi mucosa resulted from degenerative necrosis. These deleterious histological changes were correlated with thickening of sub mucosa, in addition there were heavy poly morphoneuclear cellular infiltration in focal aggregation, glandular degeneration and necrosis, these histopathological changes indicated the histotoxic effects of AF-B1. Other sections showed slight congestion due to vascular dilation indicated the tissue ischemic injury resulted from hypoxic hypoxia. On the other hand, analysis of light microscopic photograph of ileum sections obtained from rats received propolis showed no pathological changes. Furthermore some sections showed cellular infiltration of mononeuclear cells in sub mucosa specially around blood vessels indicating a hypersensitivity reaction due to the therapeutic effects of

propolis . In addition to these beneficial effects there were intense goblet cells which characterized by a large size . In ileum section of rats received propolis with AF-B1, there were cellular infiltration nearby dilated capillaries , which indicated an immune response.

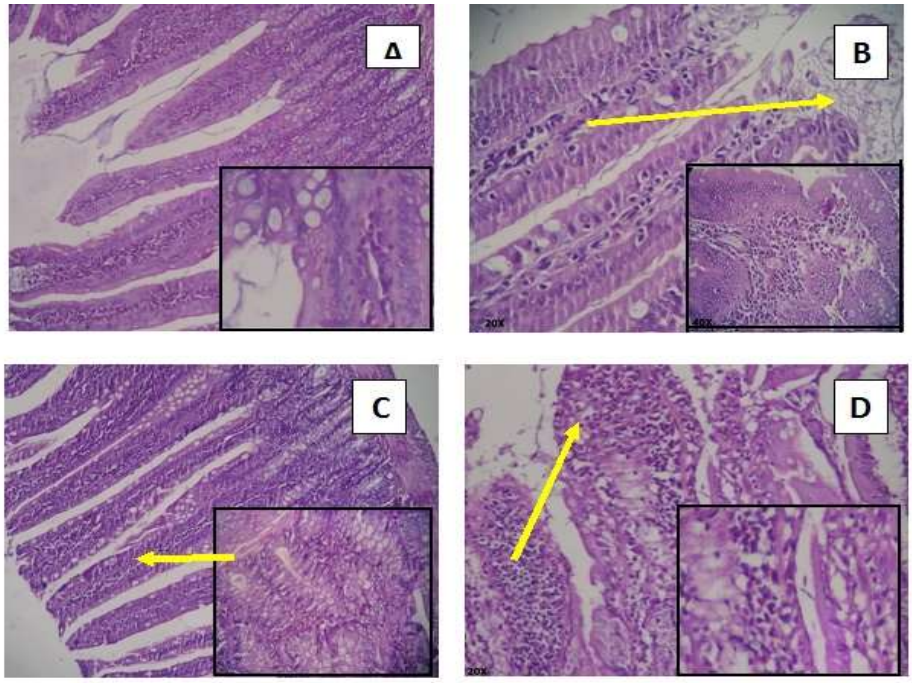


Figure -5 : Light microscopic photograph of intestinal wall (ileum) in: A: control group, with normal appearance. B: AF group, showing superficial sloughing of intestinal villi mucosa resulted from degenerative necrosis **C: P group,** infiltration of mononuclear. **D: AFP group,** cellular infiltration nearby dilated capillaries , which indicated an immune response. **H&E.(10 &250X)**

Analysis of the light microscopic photograph of stomach sections taken from different experimental animals(figure-6) reveled that AF-B1 caused cellular depression in mucosa and sub mucosal gland. There were thickening of sub mucosa, with areas of edema , congestion , cellular infiltration of polymorphoneuclear cells and degenerative necrosis of gastric glands. On the other hand, stomach sections of propolis received group showed leukocytes infiltration in nodular like structure correlated with an increase in new vascularization of the tissue due to an increase in number of capillaries with no signs of congestion. Other sections showed an increase in the mucus secreting cells. The same beneficial effects of propolis were found in stomach sections of rats received propolis with AF-b1resembeled by sub mucosal cellular infiltration in addition to goblet cells hypertrophy as well as sub epithelial intense cellular monolymphocytes.

In the present study the intestine was the organ most affected by Aflatoxin-B1 toxicity than stomach did, because aflatoxins are inhibited by the acidic PH of gastric juice and reactivated by the neutral PH of intestinal juice , especially in the duodenum where there is pancreatic excretion[15]. The intestinal histopathological chang-

es can be attributed to different effects, some of them are direct, such as toxic effects of aflatoxin in generating intracellular ROS in a quantity exceeds the cellular antioxidant capacity [30] results in membrane lipid peroxidation, and cellular damage. Disruption of junctional proteins ,and impairment of intestinal epithelial barrier integrity attributed to Aflatoxin-B1[16,27] . In addition to be the organ that play an active role as physical barrier intestine, it is an important component of mucosal immune system that can vigorously affected by Aflatoxin-B1 [13, 40] . Furthermore, the anemia caused by aflatoxin may bee influences the normal intestinal architecture in indirect manner, because anemia causes hypoxic hypoxia , tissues ischemic injury, and resulting in the sub mucosal congestion [1] . All these deleterious effects of AF-B1 on intestinal mucosa were improved in AFP and P groups. Dietary supplementation with natural material protect intestinal mucosa from Aflatoxin-B1 induced impairment, such as Selenium, probiotic bacteria [25] ,Yeast cell wall (YCW) preparations[40]. On the other hand, propolis showed highly effectiveness in counter act the toxic effects of other toxins, like Aluminum on intestinal mucosa [6] and on the thyroid function [3] . Propolis contains many chemical active substances like flavonoids and caffiec acid that make propolis be an antioxidant [29], antimycotoxins [21], and improve immune system[12] . Specifically, water propolis extract preserved intestinal integrity in irradiated rats [30].

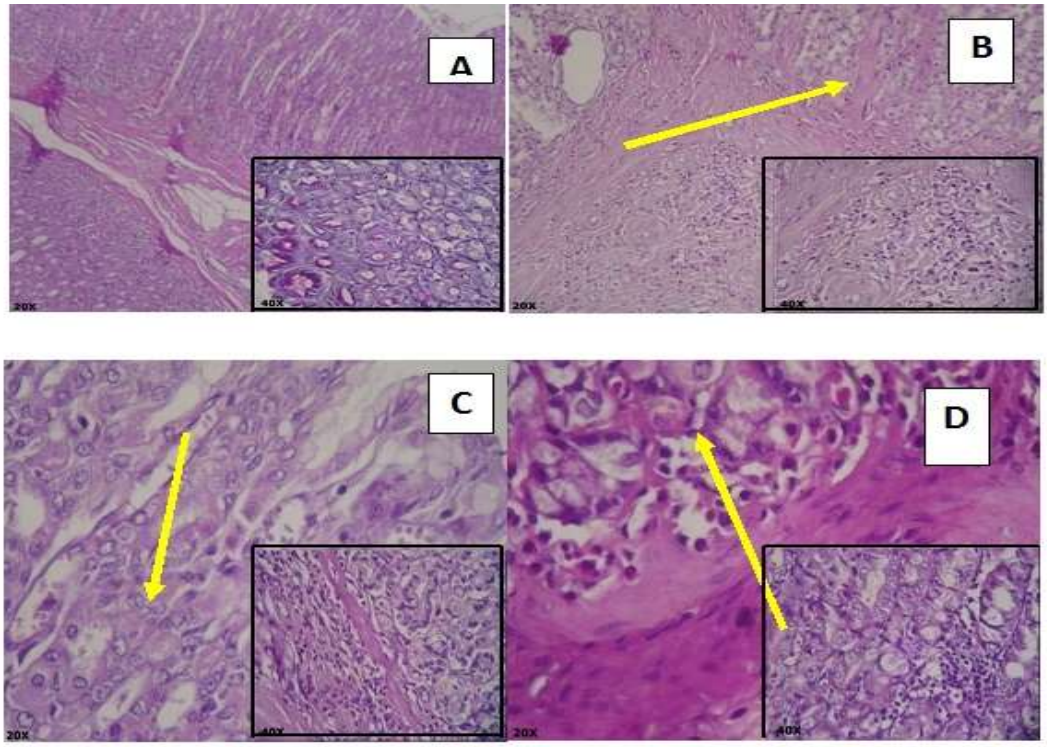


Figure -6 : Light microscopic photograph of stomach wall in: A: Contr group. B: AF group. C: P group. D: AFP group H&E.(10 &250X)

Conclusion The result of the present study concluded that Oral exposure to Aflatoxin-B1 for 60 days induced alteration in intestinal histomorphological measurements, D-xylose absorption and caused histopathological changes , indicated the direct and indirect (tissue hypoxia) harmful effects of AF-B1 on GIT specially the duodenum. In addition Propolis showed a high activity to improve these alterations to make it suggested as a good candidate for prevention and counter the deleterious effect of AF-B1.

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